

A STUDY OF THE GENERAL BIOLOGY, MOR-
PHOLOGY OF THE RESPIRATORY SYSTEM,
AND RESPIRATION OF CERTAIN AQUATIC
STRATIOMYIA AND *ODONTOMYIA*
LARVAE (DIPTERA) *

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INTRODUCTION

IN RECENT years very little has been done, especially in this country, on the biology of *Stratiomyia* larvae. The excellent publications of Swammerdam (1737-38; 1758), Réaumur (1738), and Miall (1895) on the aquatic form "*Stratiomys*" *chamaeleon* were so thorough and so excellent in quality that, evidently, few investigators thought it worth while to attempt further study. However, the workers mentioned above did not exhaust the possibilities for research in the field of either the morphology or the physiology of these insects. The taxonomy of stratiomyiad adults is now in a relatively satisfactory condition, but the classification of the larval stages is still quite imperfect.

The peculiar habits and structures of certain aquatic stratiomyiad larvae make them especially favorable for the study of respiration. Larvae are generally abundant and cosmopolitan in regions where fresh-water ponds and slow-running streams abound. Under conditions of normal rainfall larvae occur in great numbers in the small lakes and ponds, and in the Huron River in the vicinity of Ann Arbor, Michigan.

A thorough morphological study of the tracheal systems, spiracles, and accessory respiratory structures of several species was necessary before engaging in experiments on the physiology of respiration. An attempt was made in this investigation to determine the method of respiration of these larvae by both morphological and experimental

* Contributions from the Department of Zoölogy of the University of Michigan.

approaches. In addition to the major work on the structure and function of the respiratory system of these larvae, numerous data on their general biology have been secured. The results included in this paper were obtained during the period from March, 1928, to March, 1932.

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HISTORY

Early records of the Stratiomyiidae date back to the work of Aristotle. Very early these flies were confused with the tabanids. Aristotle¹ recognized the two groups as distinct, and Mouffet (1658) rails against the authors who confuse stratiomyiads with *Tabanus*, but this confusion apparently persisted for another century. In the classic, *Bybel der Natuure*,² by Swammerdam, an attempt is made to establish the name "*Musca asilus* or Gadfly" for the common European "*Stratiomys*," thus distinguishing it from *Tabanus*. This splendid work deals with the general biology and the external and internal morphology of the larva, pupa, and adult of "*Stratiomys*" *chamaeleon*. Special emphasis is placed on the structure of the digestive and respiratory systems and their transformation during metamorphosis. In general, the accuracy of Swammerdam's observations is astounding, considering the circumstances under which the work was done. His contemporary, Réaumur (1738), independently made almost the same observations regarding the habits and structures of these flies. Réaumur's plates indicate that a number of species representing several genera were studied. This work stresses a classification of his

¹ *Historia Animalium*, 5:19, 551 b; 8:10, 596 b. In the Oxford translation of this work a note on the former reference distinguishes between the tabanid and the gadfly.

² In the following pages all references are to the 1737-38 edition of Swammerdam's work.

own making. From time to time data on the natural history of these flies were added until Miall (1895) devoted a chapter to "*Stratiomys*" in his *Natural History of Aquatic Insects*, and Hart (1895), in this country, made a splendid contribution to the life-histories of a number of the common species of *Stratiomyia* and *Odontomyia* from the Illinois River. Henneguy (1904) and Berlese (1909) copied the figures of Swammerdam and others without adding original information. Miall (1895) included some of Swammerdam's figures; Hart (1895) used only original figures.

With regard to the name *Stratiomyia* the following record is of sufficient interest to be mentioned. Geoffroy (1762) first established the genus "*Stratiomys*." Osten Sacken (1878) claims that Geoffroy, in translating Réaumur's "*Mouche armée*," evidently intended the name to be *Stratiomyia* and not "*Stratiomys*." Loew (1865) amended the genus name "*Stratiomys*" to *Stratiomyia*, which still prevails.

METHODS

COLLECTION

Collections were made with a long-handled dip net, or the material in which larvae were known to be present was sorted by hand. Sometimes it was very profitable to skim the surface of a pond or the margin of the river with the dip net and take to the laboratory the débris containing the larvae. At other times it was most convenient to sort the specimens from the débris in the field.

The larvae of *Odontomyia cincta* Olivier and *O. virgo* Wiedemann occur at the surface of shallow pools and margins of streams or crawl about in damp moss, loose bark, and decaying wood, and also in débris of dried-up ponds. *Stratiomyia discalis* Loew, *S. norma* Wiedemann and *S. lativentris* Loew were found in the muck along the shore of the Huron River below the outlet of a city sewer. The larvae of *Stratiomyia* seemed to prefer abundance of decaying organic matter well shaded by cat-tails and other plants.

Moss and débris brought into the laboratory were allowed to dry. Reduction of moisture caused the larvae to crawl about freely, thus making them easily seen.

CULTURE

When larvae of *Odontomyia cincta*, *O. virgo*, *Stratiomyia discalis*, *S. norma*, and *S. lativentris* were brought in from the field along with

habitat material, cultures were made, in containers of various sorts, to correspond as nearly as possible to the conditions in nature. Tap water, cistern water, and water from the source where the larvae had been collected were used separately in these cultures. Glass covers prevented the escape of larvae, and ventilation was effected by placing a towel under the cover.

In these cultures larvae grew and pupated, and adults emerged. Larvae were also isolated in small, covered stender dishes, some with water and others without water. Usually, the last larval instars were selected for isolation in order to obtain the final exuviae when the imago emerged. It is important that only very little water be in these small containers at ecdysis. Excess moisture has no deleterious effects on ecdysis itself, but the newly born fly is often permanently maimed by getting wet. The wings inflate and harden best in a dry circulating atmosphere. Wings that happen to get thoroughly wet before complete inflation seldom are perfect when dry. Sometimes, too, mold develops about the anal and respiratory orifices. This infection is usually fatal. Occasionally most of the larvae in a given locality are destroyed by parasitic Hymenoptera.

Larvae will thrive for months in foul débris, in fact, as long as they get air for respiration and plenty of food. Although they respond to light and temperature, they are not seriously affected within the limits of ordinary laboratory conditions. It is possible to keep the last instar larvae without food from late fall to the following spring. One larva of *O. cincta*, taken on October 8, was kept, on the writer's desk, in water with a small branch of *Chara* until April 18, when a perfect fly emerged. The *Chara* disintegrated slightly, and water was added several times. For weeks at a time this larva was not observed to move from its position in the stender dish. Although the laboratory was warm throughout this period, the larva rested on the bottom, completely submerged. When picked out of the water, it would slowly bend to a crescent shape, drawing the tip of the abdomen up toward the head. It would very slowly straighten out when returned to the stender dish and remain on the bottom as if dead. At the time of pupation it came to the surface of the water and stayed there until metamorphosis had been completed. Swammerdam's figures of the characteristic shape of the larval skin inclosing the pupa made it possible easily to distinguish the pupa from the larva.

All attempts to have these flies mate and oviposit in the labora-

tory were futile. Pairs of adults were placed in a large breeding cage containing a dish filled with water. Small branches and flat pieces of wood were stood in the water and allowed to lean against the inside of the cage. It was hoped that these objects would serve as supports for the attachment of egg masses. In bright sunlight the flies were quite active, continually making an effort to escape. Although cane sugar, fresh fruits, and syrup were available for food, the flies soon died. Some, however, were seen to dissolve crystals of sugar with saliva and ingest them. This was the only feeding of imagoes observed.

TECHNIC

Microscopic technic

Material for microscopic study was prepared in several ways for sectioning. Since the tough, hard integument of the larvae resisted the penetration of fixing fluids, clearing oils, and embedding materials, work was done, not with the larva as a whole, but with individual, excised somites. Tissue embedded in Johnson's special India rubber, paraffin, and asphalt mixture (1903) sectioned much better than in pure paraffin. Even with the added toughness due to rubber in the block containing the tissue perfect serial sections were seldom obtained.

The best results were obtained by fixing individual, isolated somites of larvae in Carnoy and Lebrun's fluid (Lee, 1928), and embedding in paraffin, the melting point of which was 48-50 degrees centigrade. Cedar wood oil was employed for clearing the tissue. Long staining in a weak solution of magenta red gave excellent results, which, however, were of a temporary nature, since the color is fading in all sections. Serial sections were cut 5 to 20 microns thick.

Imperviousness of the skin was well illustrated by a larva which was submerged for 23 hours in a 5 per cent solution of sulphuric acid, with no apparent ill effects. Another larva was alive after 2 hours and 25 minutes in concentrated potassium hydroxide. To remove calcium salts, which often impregnated the integument, live larvae were dropped into 2 per cent hydrochloric acid until effervescence ceased. A bath in tap water was followed by submerging them for 20 minutes in 10 per cent warm potassium hydroxide to soften the chitin. The acid and hydroxide stimulated activity on the part of the larvae, but apparently caused no injury.

*Gross technic**Stained oil dissections*

Attempts were made to fill the tracheal system of larvae with stained oils for the purpose of making the small tracheae more readily visible. Like Hacker (1925), the writer obtained the best results with kerosene plus Sudan III and with xylol plus Sudan III. Vegetable oils, such as olive oil, were much too viscous. These stained oils entered the posterior spiracles and partly displaced the gas in the tracheal system. Complete displacement never occurred in a single specimen. However, by combining the results from all the larvae subjected to the stain a composite whole of the tracheal system was made. Living larvae were submerged in the two oils mentioned until after activity had ceased. This method of making the tracheae apparent with stained fluids was effective only with young larvae. Larvae in the last instar have perfect control of the respiratory chamber, so that stained oil does not enter in sufficient quantity to be useful. Since all the morphological studies were made of mature larvae, a more satisfactory technic was necessary to render the tracheae visible.

Glycerine dissections

Wahl (1900) injected pure glycerine into the haemocoel of living *Eristalis tenax* larvae with good results. The writer, instead of injecting glycerine into the body cavity, made a series of punctures in the integument and then submerged the larvae until the body fluid had been replaced by glycerine. To relax the larvae before submerging them concentrated chlorotone was injected with a very fine hypodermic needle into the haemocoel dorsad to the ganglia. The tracheal system remained fully inflated with gas while submerged, and could be preserved in this condition for future dissection.

Dissections were made in shallow stender dishes half-filled with paraffin. The paraffin provided (1) a suitable surface upon which to pin specimens for dissection; and (2) a white background for making photographs. Larvae were held in place for dissection by means of small sharp pins passed through the head and last somite. After the specimens were relaxed, they were always kept submerged in glycerine.

Gross dissections were made to show the dorsal and ventral

aspects of mature larvae of both *Stratiomyia* and *Odontomyia*. In either exposure right and left lateral incisions were made through the integument from the head to the last somite. A transverse incision joining the two lateral incisions was made back of the head. While the cut edge of the body wall, produced by the transverse incision, was gently lifted with forceps, the tissue beneath was slowly separated from the body wall with a small knife. As this operation progressed pieces of the freed wall were snipped off until the upper surface of the thoracic and abdominal regions had been completely exposed. The integument of specimens that had been thoroughly infiltrated with glycerine separated very readily from the soft tissue beneath without disturbance or injury to the finest tracheae. If the tracheal trunks or tubes were cut or even slightly punctured, glycerine slowly entered and gradually filled the system; otherwise, they remained full of gas.

The method of preserving these dissections was discovered by accident. Three dissections were made on September 22, a very warm day. Uncompleted observations made it necessary to keep them for subsequent checking. Plenty of glycerine was added to the specimens, and they were put into an electric refrigerator at a constant temperature of 5 degrees centigrade. The visibility of the tracheae has remained perfect in two specimens for a period of more than twenty-seven months.

Photographic technic

Motion pictures of active young larvae in water, photomicrographs of the plumose hairs of anesthetized larvae and dissections of the tracheal system, and large photographs of dissections were made. A Sept motion picture camera was employed, which used an Eastman film 35 mm. in width. Exposures were made through the side of a glass jar filled with water which contained the swimming larvae. The results and purpose of these pictures will be discussed later in this paper. Pictures of the plumose hairs were obtained by photographing *Odontomyia* larvae which had been anesthetized with either chloroform or ether. A Zeiss "Phoku" photomicrographic ocular was used on a compound microscope. Each larva was suspended at the surface of the water by its corona of hairs. In spite of the reflection of light from the water a series of good profile views of the posterior end of the larva was obtained by transmitted light.

BIOLOGY OF THE LARVAE

HABITATS

Representatives of the two genera of larvae discussed in this paper were collected in two distinct habitats. *O. cincta* and *O. virgo* were obtained in small shallow pools in hardwood forests and in sphagnum of the tamarack zone of a swamp. These larvae thrive in open water partly shaded either by trees on the bank or by aquatic plants. When the pools dry up during the summer the larvae crawl into the moist bottom muck, under bark of fallen trees and branches, and in moss covering the shore and objects normally submerged. The water in these pools usually becomes foul from decomposition of organic matter and often completely disappears through evaporation. During periods of drought and throughout the winter these larvae remain in moist, well-aërated situations. In the pond habitat they move freely from open water to moist débris on the shore, or from the shore into the water. In the swamp both species were taken in the damp sphagnum covering stumps, fallen branches, and small hummocks.

The Huron River provided an entirely different situation. Owing to a dam, water level in that part of the river where collecting was profitable remains constant. The current is very slight; abundant sewage waste fouls the water and collects along the narrow littoral region. In the mud and decaying organic matter of this narrow zone the larvae of *Stratiomyia discalis* Lw., *S. norma* Wied., and *S. lativentris* Lw. occurred. Specimens were most plentiful in the muck and débris among the cat-tails and other plants which furnish shade. Partly submerged pieces of wood covered with débris were common situations for these species.

ADAPTATIONS

Larvae of *Stratiomyia* and *Odontomyia* used in these observations are somewhat amphibious, since they can live for days at a time either submerged in water or entirely without water. They are air breathers with spiracles and an extensive system of tracheae. When a larva is at the surface the exchange of gas with the atmosphere is quite regular, but when it is submerged it must draw on the oxygen supply already in the tracheal system, as will be shown in the discussion of respiration. The great volume of gas held by the tracheal trunks is

adequate for long periods of time, especially when the larva is inactive and the temperature is low. In the absence of water this same system functions perfectly. Adaptation of the tracheal system to permit extended periods of submergence is certainly advantageous to air-breathing larvae which are aquatic feeders.

The single pair of spiracles situated at the bottom of the respiratory chamber and completely inclosed by the body wall of the last somite is splendidly adapted for screening air and preventing the passage of liquids into the tracheal trunks. In conjunction with the respiratory chamber and its orifice the spiracles form a morphological adaptation for insuring the admission of air free from foreign particles and the exclusion of undesirable fluids. Since these larvae live in habitats where accidental submergence is quite likely to occur, and where débris and disintegrating materials are prevalent, these two adaptations are very important.

Swammerdam described the integument as of a shagreen texture. The epidermis consists of hexagonal, calcareous cones, the apices of which extend into the laminated dermis. Miall (1895) ascribes the flexibility of the skin to the slight bending between these cones. The calcareous cones give a hard surface to the larval skin, and the dermis is responsible for its toughness and strength. The skin is much thinner in the intersegmental regions, where the greatest movement occurs. The remarkable adaptation of the integument lies in its pronounced impenetrability, both to sharp piercing objects and to fluids. It has long been known that "*Stratiomys*" larvae are not readily killed in "spirits of wine," and it has already been mentioned that larvae can endure sulphuric acid and potassium hydroxide for hours without fatal results. The author used 70 per cent alcohol to preserve larvae, and discovered that many survived after a period of twelve hours' continuous submergence. Larvae may crawl about in ether or chloroform for several hours without being anesthetized sufficiently to prevent muscular contraction during dissection. When these reagents do affect larvae, it is quite probable that they enter primarily through the openings to the digestive tract and respiratory system, and not through the skin.

With such a substantial covering these animals apparently have few hazards from small predatory enemies, floods, or droughts. Representatives of several genera of parasitic Hymenoptera are known to attack young larvae.

LOCOMOTION

Swammerdam mentioned that the "worm" of "*Asilus* carries its legs within a little snout near its mouth." The structures mentioned are a pair of palps, which lie in clefts on either side of the ventral surface of the head. They are armed with hooks and hairs and function both in locomotion and in feeding. The palps vibrate almost continuously, even when the animal is suspended from the surface film. These structures are not legs but mouth parts, used as accessory locomotor organs.

In crawling on a firm slightly rough surface the caudal end of a larva remains fixed while the body is extended, carrying the cephalic portion forward; then the cephalic end remains fixed in position while the body contracts, bringing the caudal portion forward. The palps and the rostrum anchor the cephalic end while the caudal portion of the body is brought forward. Hooks and hairs on the ventral surface of the abdominal somites anchor temporarily the posterior portion while the anterior portion progresses. Some *Odonomyia* larvae have strong hooks on the distal rim of the sixth and seventh abdominal somites, but not on the eighth somite; others have weaker hooks on the fourth to the seventh somites inclusive. These hooks are directed forward and are of use in maintaining the position of the posterior portion of the crawling larva.

On a smooth surface larvae progress by rolling and squirming. Since the greatest diameter of the body is in the region of the meta-thoracic and first abdominal somites, the larva will not roll like a perfect cylinder; thus contortions of the body, due to contraction and bending, give a rather undirected course of progression. However, larvae placed on glass, under the heat and light of an electric bulb, manage to get away from the spot of greatest discomfort by rolling and squirming.

Locomotion in water consists of moving about on the bottom and over submerged objects, rising from the bottom, and swimming at the surface.

The specific gravity of these larvae is quite variable, usually being less than that of water. They seem to be able to change their specific gravity to suit their needs, for they move about over the bottom and submerged objects or rise to the top at will. Beneath the surface they progress with a smooth gliding movement, owing to the

rapid alternating vibration of the palps. The center of gravity is in the anterior end and keeps the head down just enough to allow the palps to vibrate on the substratum and move the body. It was the observation of this type of locomotion that led Swammerdam to refer to palps as legs.

Ascent to the surface (text Fig. 20) is a flotation process related to respiration and will be discussed in the experimental section of this paper. It is sufficient to say here that larvae resting below the surface expel from the respiratory system gas which collects as a large bubble and is held by the plumose hairs on the tip of the abdomen. Eventually, the bubble becomes large enough to float the larva to the surface. While rising from the bottom the larva generally assumes a sigmoid shape, with the posterior end uppermost. It may wiggle as it slowly rises.

Swimming at the surface involves both the effect of surface tension upon the caudal plumose hairs and the irregular movements of the body. With the corona of hairs spread funnel-like on the surface the surface tension is great enough to float the larva with the body suspended, head downward, at various angles. Progress is made by a slow ventral flexion of the body followed by a sudden twist, which turns it completely around. These movements are repeated, with the larva turning sometimes to the right and at other times to the left, and they cause it to move along an irregular course. In spite of the sudden twist the corona glides at the surface. These larvae, especially the young ones, are very supple. Their movements in water are exceedingly graceful when compared with the clumsy crawling method of locomotion on solid substratum.



FIG. 20. Diagram of a larva with a gas bubble, rising to the surface of the water

FOOD

Swammerdam found that "clay and soft earth are the food of this insect." He was puzzled by the presence of "little red stones

and small grains of sand" mixed with the digestive products in the intestine. From the size of the mouth he was sure that they had not been swallowed and concluded that they had been formed from the food sucked into the stomach. Miall (1895) makes one brief statement: "... the food consists of microscopic organisms swept into the mouth by the palps." Hart (1895) mentions *Stratiomyia norma* Wied. as "seeming to browse upon the minute life" occurring on branches of *Ceratophyllum*. The stomach contents of one larva of the species *Odontomyia cincta* "seemed to be mostly mud with a little vegetable matter, and here and there a diatom frustule." The author has observed both young and mature larvae feeding on the soft tissue of dead leaves, from deciduous trees, that had dropped into the pool at least one season before. In the laboratory larvae would clean the soft tissue from a leaf, leaving nothing but the venation. Soft, decaying wood and bark are often favorite food materials in the natural habitats as well as in cultures. In one particular instance the soft wood between the hard annual rings was eaten out by larvae of *O. cincta* and *O. virgo*. It is quite probable that bacteria and other minute organisms, both plant and animal, are also food for these larvae.

RESPIRATION

Since respiration of stratiomyiad larvae is one of the major divisions of this work, it will be discussed under the heading "function of the respiratory system."

OVERWINTERING

The mature larvae of *O. cincta* and *O. virgo* have been found after the snow and ice had melted from the ponds in spring. The taking of small young larvae, presumably of both species, at the same time indicates that larvae in both stages of development overwinter in this latitude. Hart (1895) states that a large percentage of the larvae of *O. cincta* collected in December, February, March, and April, along the Illinois River, were quite young.

The presence of larvae in the water as soon as the ice thaws leads one to believe that they spend the winter in the pools or very near to them. The last larval instar of *O. cincta* and young larvae of *O. virgo* have been found in moss and debris above the water level of pools and swamps. Scores of *O. cincta* were collected in sphagnum

on November 10, 11, and 20, 1930, and again in the same situation on April 2, 1931. Freezing weather and snow prevented later collecting in November. On April 2 only the upper layer of sphagnum had thawed, an indication that the temperature in the moss had not risen much above freezing. Inactive larvae were found in the moss above the ice in identical places where other larvae had been collected the previous November. There is every reason to believe that these larvae had not moved during the winter, but hibernated in the sphagnum.

Where the moss was thick, the larvae generally occurred one or two inches below the surface in loose, well-aërated, and moist situations above normal water line. A thin covering over logs, stones, or the shore itself often harbors larvae. When compact and water-soaked, moss was less frequented than when loose and aërated. In several instances specimens were found frozen in débris among the surface irregularities of rotten wood covered with moss. In November *Stratiomyia* larvae were found in gravel, six inches above the water line of the Huron River. The situation was moist, but not very wet. Although there had been freezing weather the larvae were not frozen.

Larvae survived freezing of the cultures containing them, both in refrigerators and on the window sill of the laboratory. The exact temperature was not obtained, but it was low enough to congeal the débris and water. One might well conclude that in both young and mature stages these larvae may spend the winter above the water and that they can survive exposure to temperatures below freezing.

EXCRETION

Since the time of Swammerdam it has been known that the larvae of "*Stratiomys*" possessed four "vascula varicosa," or caeca, containing milky white fluid. These caeca have been identified as Malpighian tubes, and their function has been determined as excretory by subsequent investigators. Vaney (1900; 1902) found calcium carbonate in the tubes of "*Stratiomys*" larvae. For a number of years there was some question whether the anterior pair of tubes, or the posterior pair, or both pairs, did the secreting. Pantel (1914) claimed that he had found calcium carbonate in all the tubes and that it is an ordinary product of excretion. Keilin (1921) confirmed

the work of Vaney and Pantel with reference to excretion of calcium carbonate by the Malpighian tubes of *Stratiomyia* larvae and suggested that it might be a product of respiration.

While working with the larvae of *O. cincta* the author observed that the Malpighian tubes contained a number of substances varying from a milky white fluid to large, solid concretions, which caused the walls of the tubes to stretch beyond their normal diameter. The tubes of some animals contained an amorphous, chalky material, either white or tinged with yellow, and occasionally extending into the hind gut. Other specimens had opalescent bodies resembling pearls scattered in the tubes in a haphazard manner. These were apple-shaped, almost spherical, or hemispherical. Individual concretions varied a great deal in size, and frequently numbers of them were firmly cemented together, resembling in arrangement a cluster of grapes. These opalescent individual concretions and clusters resisted pressure applied with a dissecting needle as if they were glass beads, and crushed with distinct cleavage. They are heavy enough to collect in the bottom of a dissecting dish while the débris is removed and the water decanted. Opalescent concretions occur in the hind gut as well as in the tubes.

In addition to the substances already mentioned crystals were found both in the tubes and in the body cavity. Those in the tubes formed plugs which filled the lumen and usually were two or three times as long as their diameters. These cylindrical plugs had rounded ends, and the sides were corrugated parallel to the long axis. They consisted of a series of slender prismatic crystals arranged in palisade formation. These crystalline plugs were translucent and varied from straw color to brown. In one instance two plugs were separated by soft, white, noncrystalline material. In addition to these plugs small, square, transparent crystals occurred both in the tubes and in the haemocoel. These were hard and clear, like glass.

The fact has been quite well established that insect larvae which feed on putrefying material produce large quantities of calcium salts which are dissolved at the time of pupation and at least partly reformed prior to ecdysis. The reformed products are left in the puparium. However, not all the secretions of the Malpighian tubes are retained and dissolved during metamorphosis. The larvae of *O. cincta* have been seen to void milky white excreta. It could not be confused with the dark fecal matter and, when treated with

hydrochloric acid, it effervesced, liberating carbon dioxide. Voiding of other excretory products has never been observed, although a number of small opalescent bodies, like those common in the tubes and hind gut, were found in a culture dish containing active larvae. There is no doubt but that they had been eliminated through the anal aperture.

Not all larvae collected in the same pool and at the same time contained concretions. Adults were not examined, but both pupae and larvae possessed them, especially those larvae that had overwintered. One larva collected in July contained more than 186 individual concretions as well as chalky material. Many were cemented together, which made definite counting impossible without destroying the clusters. They were the translucent, opalescent, and straw-colored varieties, and they ranged in diameter from 0.04 to 0.5 mm. inclusive. The larva taken in July was about ready to pupate when it died.

A number of tests were made to determine the carbon dioxide content of the gas produced when concretions were treated with hydrochloric acid. The methods employed by Krogh (1908) were used in making these determinations. Although the series of tests was small, the results were diverse. One concretion produced gas which contained 88.2 per cent carbon dioxide. In another test three concretions from the same larva produced gas of which 56.2 per cent was carbon dioxide.

METAMORPHOSIS

Stratiomyiidae are a border-line family between Nematocera and Brachycera. They are classed in Brachycera because of the antennal structure of the adults, but Miall (1895), speaking of "*Stratiomys*," says: "It is the simplest in structure and life-history, and the most like a Nemoceran, of all the Diptera which retain the larval skin as the outermost covering of the Pupa." Réaumur (1738) and Swammerdam described pupation and ecdysis in species belonging to the family Stratiomyiidae. Swammerdam says that he has "often seen this worm in the space of twelve hours . . . change into a nymph."

The time required for the change from an active larva to a pupa is variable. The mature larva gradually becomes inactive and rigid. Inactivity is followed by a downward bend in the intersegmental region between the fifth and sixth abdominal somites and an upward

bend in the intersegmental region of the seventh and eighth abdominal somites. These permanent flexions indicate that pupation has begun. The pupal stage lasts but a few days when the weather is warm. During the pupation period the animal becomes much smaller and occupies only part of the cavity in the larval skin. This newly formed pupa pulls loose from the body wall at the caudal end, leaving the respiratory chamber and the posterior spiracles in position, and vacating the fifth, sixth, seventh, and eighth abdominal somites, which fill with air. In the anterior end most of the first thoracic somite is vacated, and it, also, fills with air. These two pockets of air within the skin serve to float the inclosed pupa and provide a pneumatic raft for the imago when it emerges.

When the larva shrinks from the integument to form the pupa the lining of the hind gut remains attached to the larval skin. In the anterior end the spiracles with portions of the tracheal trunks and the pharyngeal lining are retained, along with the head capsule and cephalic appendages. A thin membrane which covers the pupa is left in the larval skin when the adult emerges. This pupal skin contains parts of the tracheal system. As the pupa develops the head and thoracic regions swell, inflating the puparium to its limit. Transformation from functional larval structures to organs and systems for the adult is rapid.

The puparium eventually bursts sagittally along the dorsal surface of the mesothoracic and metathoracic somites and part of the first abdominal somite. At each extremity of the sagittal slit a transverse slit occurs. Through this I-shaped opening the imago emerges, leaving the pupal exuviae in the larval skin. Ecdysis may take place either from a floating puparium or from a perfectly dry one. The process of emergence, hardening of the body, and inflation of the wings have been described in detail by Réaumur (1738) and Swammerdam.

MORPHOLOGY OF RESPIRATORY SYSTEM

The *Stratiomyia* and *Odontomyia* larvae used in the investigation of the morphology of the respiratory system have metapneustic respiratory systems. Two main tracheal trunks extend the length of the body, terminating cranially in a small pair of dorso-lateral spiracles on the prothoracic somite, and caudad in a pair of large spiracles situated in the respiratory chamber in the last abdominal

somite. There is no experimental evidence that the anterior pair of spiracles is functional. Long lateral trunks, smaller in diameter, laterad and parallel to the main trunks, arise near the cephalic end of the main trunks and terminate in them at about the middle of the last somite. Commissures and tubes connect these tracheal trunks to form a more or less double system for the circulation of gas.

The respiratory chamber, which contains the posterior spiracles, is situated in the last abdominal somite. The orifice to it is a transverse slit at the tip and is surrounded with plumose hairs. Swammerdam, Miall (1895), and Hart (1895) all claim that, with the exception of the mesothoracic somite, each body somite has a pair of spiracles. Miall adds that "branches from the longitudinal air tubes pass to them on the inside." The question of whether these larvae have more than two pairs of spiracles will be discussed later.

RESPIRATORY CHAMBER

Since the main opening to the respiratory system is in the tip of the last abdominal somite, the structures of this region will be described first. The respiratory chamber is a membranous sac completely inclosed by the body wall of the last one fourth of the somite. Entrance to the chamber is through a transverse orifice in the end of the abdomen. Accessory structures consist of plumose hairs surrounding the orifice and a pair of air sacs extending cranial from the floor of the chamber. Each will be described in detail, separately.

Morphology of plumose hairs

The plumose structures (Fig. 23 *ph*) surrounding the respiratory orifice have been called hairs by Swammerdam and bristles by Wahl (1900), when referring to similar structures in *Eristalis tenax* L. Henneguy (1904) and Berlese (1909) both called them "rigid, chitinous filaments" forming a "caudal corona" in "*Stratiomys*" *chamaeleon* larvae. Dunavan (1929), working with *Eristalis arbustorum* L., called them "feather-like setae." The terms "hairs," "bristles," and "setae" have been used promiscuously for this circle of structures attached to the dorsal and ventral rims of the opening to the respiratory chamber. According to Imms (1925), these structures should be classified as "plumose hairs," under the broad term "setae." The mature structures conform to the definition of hairs and not of bristles. However, since the development of the Stratio-

myiidae has not been traced from the embryo through the various instars of the larva, this matter cannot be settled positively at this time.

These plumose hairs occur along the crescent-shaped dorsal and ventral rims of the tip of the abdomen just back of the chitinous blades which guard the orifice to the respiratory chamber. They vary in number, as indicated in the following table:

TABLE I
NUMBER OF PLUMOSE HAIRS IN CAUDAL CORONA

Species	Hairs in dorsal rim	Hairs in ventral rim
<i>O. cineta</i>	24	40
do.	27	36
<i>O. virgo</i>	24	40
do.	26	36 +
do.	28	28
<i>S. norma</i>	23 +	22 +

The hairs of *O. cineta* are most readily counted although very small hairs, sometimes interspersed among large ones and near the ends of each crescent, are easily overlooked. It is very difficult to count hairs on *S. norma* because the long ones are few and the small ones abundant. There is a marked tendency in some species for these hairs to occur in a series of tufts, each tuft consisting of long, large hairs separated by one or more small ones. The hairs on the ventral side, especially in *O. cineta* and *O. virgo*, are arranged in three such tufts. The grouping into tufts is less marked on the dorsal side. The longest hairs are in the middle of each tuft, and all together form the apex of the cone, whereas the short and intermediate hairs give increased circumference to the base and body of the cone.

These hairs vary in length from a small fraction of a millimeter to a maximum of 1.6 mm. The proximal end of the shaft of a hair is comparatively stout, but tapers gracefully toward the distal end. This end also has a distinct curve with the concave side facing the orifice. Each hair is firmly attached at the base, but the shaft is flexible enough to assume the shape of the surface of the water or

to curve about the terminal bubbles of gas. Plumules occur along either side of the shaft from a position near the place of attachment to the extreme tip. Those near the base are short, but they gradually increase to a maximum length which is maintained almost to the tip. The length of the plumules is about equal on both sides of a shaft. The maximum length recorded was 92 microns.

Plumules arise on the somewhat flat side of each hair and diverge from both sides of the shaft. Those on one side are directly opposite those on the other. The angle of diversion is about 40 to 45 degrees while the hairs are spread out on the surface of the water and much more acute when the larvae are submerged and are inclosing a bubble of gas. When the hairs are spread, the plumules generally touch the plumules of adjacent hairs on either side. This is true throughout the length of the short hairs, but does not apply to the distal portions of the long ones. On the lateral side of the corona the plumules of the small hairs actually overlap. Under natural conditions the surface of the hairs which face toward the orifice is hydrofuge. This property is lost as conditions change.

The shaft of each hair is hollow (Fig. 20), open at the proximal end, and closed at the distal end. Near the proximal end the cavity is somewhat reduced in diameter, but terminates in a bulb-shaped space. The bulb maintains a connection with the haemocoel by a small opening in the base of the hair and by a canal through the body wall. Roughly, a transverse section of a hair is semicircular, especially the cavity. This cavity varies in shape and size in different regions of a hair, being smallest at either end and largest in the middle. The largest cavities of those measured showed a variation of 7-12 microns along the greatest diameter. As nearly as could be determined from histological preparations, this tubular cavity is filled with a fluid devoid of cells. It gives a zonal appearance as shown in Figure 20. It is not granular, but takes stain in such a manner as to give the appearance of several zones of material differing in consistency. This substance remains in sections fractured in cutting as well as in perfect sections. In fact, sections that were broken into two distinct parts retained the substance in each part.

The wall of the hair is comparatively heavy. The flat side which bears the plumules is the thicker. It consists of a layer of epidermis, a layer of dermis, and apparently a very thin layer of hypodermis, although the cells were not distinguishable. The epidermis is rather

even in thickness on the rounded side, but swollen in the middle of the flat side. It is also continuous with the plumules, extending off at angles from either side of this swelling. At the point where the epidermis thickens it replaces the underlying dermis in proportion to the amount of swelling. In addition to the extension of epidermis to produce plumules on the sides it is also projected on the rounded surface in the form of spines, hooks, and ridges. Serial sections indicate that they occur in a haphazard order. The spines and ridges are stout, and rather sharp, and are most frequent at a position (Fig. 20 *spi*) directly opposite the flat side. They may be present singly or in pairs. When single, the spine or ridge is in the middle of the semicircle, and when in pairs they are rather close to each other on either side of the mid-line. Symmetry of arrangement is pronounced. The hooks are less abundant and occur on the lateral sides of the hair.

Together, all the plumose hairs of a larva form the corona, a funnel-shaped structure when spread on the surface of the water and a cone- or bulb-shaped structure when submerged. At the bottom of this funnel or cone is the orifice (Fig. 23 *ro*) through which the air passes into the respiratory chamber. The function of these hairs will be discussed in the section on respiration.

Orifice to respiratory chamber

The orifice to the respiratory chamber (Fig. 23 *ro*) extends across the entire width of the abdomen, having hinged articulation in both right and left lateral edges. It is bounded by two heavy, bladelike bars, which form the dorsal and ventral terminations of the body wall. These blades are rather narrow, rigid, slightly beveled, generally smooth, crescent-shaped, and fit together when closed, producing a very effective seal. Ridges occur at the line of attachment to the body wall. The dorsal blade fits under the ventral one, the two overlapping each other like blades of scissors. When the orifice is closed, the beveled surfaces fit each other and the ridges mesh neatly.

These blades (Fig. 22 *bl*) consist of an exceptionally thick layer of cuticle and a thin layer of hypodermis. In some specimens a series of rounded projections were observed on the smooth surface of the mid-section of these blades. They had the appearance of small tubercles, and were arranged in series of three or four, parallel

to the curvature of the blades. So far as could be determined, these tubercles were a detriment rather than an asset to this otherwise efficient closing device.

Membrane of respiratory chamber

The respiratory chamber (Figs. 5, 24 *rc*) is a thin, membranous sac situated in the caudal end of the last abdominal somite. When inflated with gas this sac is slightly compressed dorso-ventrally and occupies most of the body cavity of that region. The blades form the periphery of the orifice and determine the size of the aperture. Two posterior spiracles are at the dorso-cranial end of the chamber, on the right and left sides. The membrane is attached to the rims of these spiracles, which are separated by a somewhat vertical strip of membrane with two small pouches directed forward. Muscles are attached to the ends of these pouches. On the floor of the chamber and ventrad to the spiracles are the openings into two large pouches. These pouches (Fig. 24 *lp*) appear to be evaginations of the chamber and lie against the ventral wall of the body, extending cranial to a position parallel to the mid-point of the anal slit. Each is connected with the chamber by a short neck which widens to form the flat, wrinkled pouches that occupy almost the entire width of the ventral side of the haemocoel. They were always partly deflated when exposed by dissection, but were readily inflated experimentally. The membrane of the large pouches is somewhat thinner and more delicate in structure than the main chamber, although it is continuous with it. At the time of ecdysis the respiratory chamber and accessories remain in the larval skin. The chamber membrane is inflated to capacity and quite firm, but the large pouches are partly deflated and wrinkled. The orifice and the spiracles are the only real openings in this chamber.

POSTERIOR SPIRACLES

The two main spiracles (Figs. 2, 5, 24 *ps*), located in the respiratory chamber, are the gateways to the tracheal system. A pair of these spiracles in *O. cincta* were 175 microns long and 140 microns wide. Viewed from the side, a spiracle resembles a truncated cone, with the base facing the respiratory chamber and the long side dorsad. They are intricate structures of firm, chitinous rings with radiating and parallel tubes, supported by membrane. Under pres-

sure they break rather than bend, but are not especially delicate for such small objects. The reaction to stain indicates that the rings and tubes are cuticular in nature.

The frame of a spiracle (Figs. 10, 11) consists of a heavy chitinous ring (r_1) into which a lighter ring (r_2) fits perfectly. This heavy ring faces the respiratory chamber. The first ring supports an earlike structure (Fig. 11 *e*) on the lateral margin, and a row of short, stout tubes which radiate to the central membrane. The earlike flap on the lateral margin is semirigid and forms part of the wall of the respiratory chamber. These erect flaps are slightly concave and often turn over along the distal margin. The surface facing the spiracle is thickly studded with coarse, slightly curved, hollow spines, which are closed at the tips and directed toward the spiracle. Some of these flaps lean slightly over the spiracles, but never have they been observed as a lid or cover of the spiracles. The median and ventral parts of the heavy ring are the thickest and support the stoutest tubes. Transverse sections through the spiracle show that this ring has a sector of chitin around the thickest part. Short, hollow tubes (tu_1) radiate from the ring to the central membrane, which occupies an irregular area inclosed by the ring. Although these tubes are not uniform in diameter, length, and intervals on the ring, they do intergrade uniformly. The base of each tube flares and is continuous with the ring, whereas the distal end flares and is continuous with the central membrane. At the base of many of the larger tubes are three small holes arranged in the form of a triangle.

The second ring (Fig. 10) consists of a lighter and somewhat smaller rim which gradually disappears on the dorsal side in the formation of a membranous lip (l). Radiating from the inner margin of this ring are tubes (tu_2) of various lengths and diameters which terminate in the central membrane (cm). These tubes are arranged in two irregular zigzag rows. The tubes (tu_3) from the lip to the central membrane do not seem to have a definite arrangement. All the tubes are hollow and flare at the ends. Those terminating in the membrane often have circular openings. In addition to the tubes attached at both ends there are hollow spines on the membrane that are free and apparently closed at the distal end.

In dehydrated and stained specimens the central membrane is wrinkled and firm. Sections show that it is thin and more than ample in area for the space which it occupies. It has never been observed

to be stretched like a drumhead and probably is soft and flexible in the living larva.

The dorsal lip is really a diffused section of the light ring (r_2). The longest tubes in the spiracle are attached to it on the inside, and the tracheal trunk is attached on the cranial margin. There are no taenidia in the lip, which makes it possible to distinguish the place where the tracheal trunk is attached.

The two parts of the spiracle just described do not separate readily. The specimens used for Figures 10 and 11 were especially good. They were obtained by seizing the ear and the lip of the spiracle with forceps and carefully pulling in opposite directions. The only apparent break occurred where the short radiating tubes in the first ring separated from the central membrane. It is believed that a spiracle is a single structure and not made up of two distinct parts. However, it is much simpler to describe it in parts than as a single unit.

TRACHEAL SYSTEM

The tracheal systems (Figs. 1-4) of the *Odontomyia* and *Stratiomyia* studied have the same general plan, namely, two main trunks and two smaller lateral trunks, all of which extend throughout the length of the body and are connected with each other by transverse tracheae. From the trunks tracheae lead off to the visceral organs, the ganglia in the thorax, the head, and the skin and muscles of each somite.

The labeling system and the terminology of Kennedy (1922), modified by Dunavan (1929), is followed wherever possible. Although Dunavan (1929) and Wahl (1900) worked on species of *Eristalis*, their sketches and descriptions were followed for the purpose of comparison of structures common to these stratiomyiads. Since there is considerable modification of spiracular and leg tracheae, a comparison of homologous parts has not been completed at this time. Figures 1-4 were made from actual specimens and are not composite drawings of a series of specimens. Until a great many specimens can be studied the homology of the lesser tracheal tubes cannot be definitely established. The variation in the number of tracheae leading to the ganglia in the thorax, as well as the variation in the origin of the tracheae, is very pronounced. These variations will be mentioned when the structures are described.

Dorsal aspect

Specimens of *O. cincta* and *S. discalis* in the last larval instar were relaxed with concentrated chlorotone and submerged in pure glycerine to prevent the escape of gas from the tracheal system. The skin was punctured to admit glycerine into the haemocoel. After six to twelve hours the entire dorsum was removed from the larva. Tracheae appeared silver-white against the glycerine background, and minute as well as gross structures were readily observed with a binocular microscope.

Main trunks

The main trunks (Figs. 2, 4, 5 *mt*) are attached to the posterior spiracles. The line of attachment follows the ventral rim and dorsal lip of the cephalic face of the spiracle. From the spiracles the trunks gradually increase in size through the abdomen and become greatly reduced in the thorax, where they terminate in the anterior spiracles and in branches to the head and pharynx. In *O. cincta* these trunks maintain a rather uniform diameter from the beginning of the seventh abdominal somite cranial to the first abdominal somite. In either the first abdominal or the metathoracic somite the trunks taper very abruptly and continue cranial to the spiracles as much reduced tubes. In *S. discalis* and *S. norma* the abrupt decrease in size of the main trunks occurs in the mesothoracic somite, and is more pronounced than in *O. cincta*. The trunks are also more slender in the sixth and seventh abdominal somites than in *O. cincta*. These trunks vary in shape with the degree of inflation. When inflated to the maximum, they approach a circular outline in transverse section, but are usually oval or flat when dissected. One inflated main trunk in *O. cincta* was one millimeter in diameter. These main trunks have taenidia which uncoil as a single thread.

It is interesting to note here that, although the general plan of this tracheal system is similar to that of *Eristalis*, the trunks are in no way convoluted to accommodate for the contraction and extension of the body. The larvae of these stratiomyiids do not contract to the same degree as *Eristalis*; nevertheless, the trunks must be very elastic to accommodate for the change in body length without convoluting. This is especially true for *S. discalis* and *S. norma*.

Dorsal connectives

Ten dorsal connectives extend from one main trunk to the other (Figs. 2; 4, 1-10). These occur at regular intervals between the mesothoracic and seventh abdominal somites. They arise in the median side of the main trunks and form dorsal loops bearing small tracheae. The capacity of these connectives is more than ample for the slender branches which emanate from them to the dorsal muscles, and might serve as viaducts for the exchange of gas from one main trunk to the other. The loop which each forms allows for the contraction and extension of the larva in locomotion as well as for maximum inflation of the tracheal trunks. Usually, four small forked branches arise at the top of each loop, two of which extend somewhat cranial and two caudad. The number and position of these branches are variable. They may have separate attachments, or two common attachments. There are from three to six branches from each dorsal connective. The first dorsal connective gives rise to extensive tracheae leading to the pharyngeal region and head capsule as well as to the dorsal muscles.

Lateral trunks

Laterad and more or less parallel to each main trunk is a long, slender tracheal tube called a lateral trunk (Figs. 2, 4 *ll*). These two trunks are attached to the main trunks anteriorly, near the anterior spiracles, and posteriorly in the last abdominal somite. In addition to the end attachments there are eight other tubes connecting each lateral trunk with its adjacent main trunk. The lateral trunks do not maintain uniformity of size throughout their length, being greatest in diameter in the same region where the main trunks are largest.

According to Dunavan (1929), the tracheal tubes connecting the lateral and main trunks in *Eristalis* are spiracular trunks (Figs. 1, 3 *a*). This is probably a very excellent descriptive name, since eight of the ten pairs of spiracular trunks are connected by small tubes to lateral spiracles. In the case of *E. tenax* L. and *E. arbustorum* the first and tenth trunks do not end in spiracles. The eleventh pair of spiracles is located at the posterior end of the main trunks, as in the stratiomyiads discussed in this work.

There is no uniformity with respect to the position and number

per somite of small tracheae from the lateral trunks to the dorsal and ventral muscles. Since these larvae have no legs the tracheae which would otherwise supply them are not needed for that purpose. Whether that accounts for the great irregularity has not been determined. The tergal tracheae (Fig. 2*t*) which supply the dorso-lateral muscles usually arise at the distal ends of the spiracular trunks. A tergal trachea may be missing, and again it may come off from the lateral trunk near its junction with the spiracular trunk. The sternal tracheae (Fig. 2*s*) to the ventro-lateral muscles usually arise on the lateral trunks caudad to the junctions to the spiracular trunks. The tergal and sternal tracheae branch and rebranch, forming numerous tracheal tubes to the muscles and the integument. Other tracheae, similar in size, arise from the lateral trunks and possibly are homologous with the leg tracheae in the larvae of other species. The ganglionic and visceral tracheae will be discussed together with the tracheae observed in the ventral aspect of the system.

Lateral spiracles

Swammerdam and Miall (1895) claim that "*Stratiomys*" possesses nine pairs of lateral spiracles. According to Miall, "branches from the longitudinal air-tubes pass to them on the inside." Hart (1895), writing about *O. cincta*, states that there are "spiracles on the upper side of the lateral margins of segments 1 and 4-10." Irwin-Smith (1923), working on larvae of terrestrial stratiomyiids in Australia, found lateral spiracles. It happens that the species of *Odontomyia* and *Stratiomyia* studied by the author have only two pairs of spiracles which are connected on the inside with tracheae, namely, the pair of lateral spiracles on the prothoracic somite and the inclosed pair in the last abdominal somite.

There are structures on the external surface of the integument which superficially resemble spiracles, but careful dissections in glycerine reveal neither tracheal tubes nor cords attached to them on the inside. To be absolutely sure that small tubes were not overlooked, serial sections were made of each somite in question.

The tissue was double embedded (celloidin followed by paraffin) and sectioned ten microns thick. Delafield's haematoxylin and eosin were used as stains. Excellent serial sections showed structures in normal position, such as tracheae, Malpighian tubes, muscle attachments, and setae with channels through the cuticle, but no spiracles

with inside connections. The brownish spot in the position of a spiracle is apparently an external indication of a muscle attachment.

Although Irwin-Smith did not make serial sections of *Metaponia rubriceps* Macquart, she concludes that lateral spiracles are present on all somites except the mesothorax. There is no evidence that either Miall or Hart made histological study of their material. It is quite apparent that the presence of lateral spiracles on the abdominal somites is not a family character, since some species possess them and others do not. In all probability aquatic larvae would be less likely to have lateral spiracles than would the terrestrial ones. Theoretically they might be regarded as lost structures, since the tendency of the primitive insects is to have eleven pairs of spiracles. However, it seems quite logical to agree with Dunavan that the tubes connecting the main and lateral trunks should be called spiracular connections.

Anterior spiracles

The anterior spiracles (Fig. 6 *as*) are located in the middle region of the dorso-lateral margin of the prothorax. Externally these spiracles are slightly oval, are composed of hyaline chitin, are somewhat elevated in the center, and have suppressed margins. The stigma is guarded by a short papilla with diameter equal to that of the stigma. These spiracles face dorsally, but beneath the integument they are directed caudad to meet the prothoracic spiracular trunks to which they are connected. Because of this bend it is impossible to get a sagittal section through the stigma, stigmatic chamber, and trachea at the same time. However, Figures 7-9 give some idea of the relative length and shape of these structures.

The stigmatic aperture (*sa*) is in the center of a cone-shaped eminence of heavy cuticle which covers the stigmatic chamber (*sc*). The stigmatic chamber corresponds to the "felted chamber" (*Filzkammer*) described by Meijere (1895). It is plugged for a considerable distance by a mesh of chitinous tubes (*tu*) which vary in size, the largest ones being near the stigmatic aperture and the smallest where the chamber joins the spiracular trunk. Although Figures 7-9 were not made from the same spiracle, they represent a typical series of sections through the stigma and chamber.

Near the stigma the largest chitinous tubes radiate from a definite point of attachment to the wall of the chamber. Figure 8 indicates that at least some tubes are open to the vestibule below the

stigmatic aperture. These tubes may extend entirely across the chamber and be attached wherever they touch its wall. In many instances it was possible to see that these tubes were open at the point of attachment. In one case in particular tubes radiated fanwise from a central tube. These tubes gradually decrease in size and increase in number, then suddenly cease to occur. They form a very intricate filter between the atmospheric air and the tracheal trunk.

Between the "felt" portion of the chamber and the taenidium-bearing trachea there is an irregular chamber with a constricted zone near the end. Its wall is thin, apparently soft and flexible. A thick layer of hypodermal cells (*hy*) surrounds the spiracular chamber and this thin-walled chamber connecting it to the main trunk.

Ventral aspect

A description of the ventral aspect of the tracheal systems of *S. norma* and *O. cincta* (Figs. 1, 3) will not include that of structures common to both dorsal and ventral aspects which have already been described, such as the lateral and spiracular trunks and the tergal and sternal branches. The principal ventral tracheae consist of (1) branches to the visceral organs, (2) tracheae to the main ganglia, and (3) the anterior commissure.

Visceral tracheae

The main visceral trachea (*b*) of a somite usually arises at the base of a spiracular trunk, but occasionally originates on some other part of it. Generally there are a pair of visceral tracheae in the metathoracic somite and a pair in each of the first seven abdominal somites. However, this may not always be the case, as is shown in Figures 1 and 3. Other variations were observed, but sufficient specimens have not been examined to determine the typical formula for these tracheae.

The minor branches (*c*) arise either at the same point as do the main visceral tracheae or directly from the main trachea slightly distad from its point of origin. These visceral tracheae have many branches and extensive ramifications.

Ganglionic tracheae

The ganglionic tracheae (Figs. 1, 3, 6 *g*) arise in the lateral trunks and end in the ganglia which are situated in the mesothoracic and

metathoracic somites. There are twelve ganglia, all but the first of which are supplied with tracheae. Each thoracic somite has a ganglionic trachea which gives off a pair of accessory tracheae to the second, third, and fourth ganglia. These tracheae (Fig. 6 *ag*) are slightly separated at their origin and end in a number of short tubes on either side of the ganglia. It should also be mentioned here that the ganglionic trachea in the prothorax gives rise to a pair of tracheal tubes leading along the pharynx.

The distribution of the ganglionic tracheae in the abdomen is puzzling. In *O. cincta*, tracheae to the ganglia occur in all but the eighth abdominal somite; in *S. lativentris*, in all but the seventh and eighth somites. *S. discalis* and *S. norma* seem to agree with *S. lativentris* in this respect. Usually one trachea per somite is produced in the abdominal region. When a pair of ganglionic tracheae are present in the abdomen they generally occur in the first somite. In *O. cincta* (Tables II and III) the variation in number is 5-8; in *S. lativentris*, 4-7. These tracheae do not seem to comply with any formula with regard to their occurrence. All may arise on one side or they may occur on both sides in many combinations.

Two facts, however, are apparent, namely: (1) that the tracheae join in the order of origin in the abdominal somites; and (2) that those which arise on the left side join the left side of the ganglia and vice versa; they have never been observed to cross from one side to the other. In *O. cincta* the usual number of unpaired tracheae is 7 and in *S. lativentris*, 6. Specimen 3 (Table II) showed the only example of a pair of tracheae in which the components united, in the sixth abdominal somite, to form a single tube leading to a ganglion.

The main tracheal trunks extend cranial from the base of the first spiracular trunks, decreasing abruptly in size as they branch. There are six pairs of branches leading to the head, atrium, and pharynx, several of which are long; the rest are rather short and blunt. One pair of the head tracheae gives rise to tubes which terminate on the lateral sides of the first ganglion; these tubes should be regarded as ganglionic tracheae similar to those already described. They are not united; nevertheless, they represent the first pair of ganglionic tracheae and will here be called the cephalo-prothoracic ganglionic tracheae (Fig. 6 *cps*).

TABLE II

TRACHEAE LEADING FROM THE LATERAL TRUNKS TO THE GANGLIA IN THE LARVAE OF *ODONTOMYIA CINCTA* OLIV.
Cephalo-prothoracic ganglionic tracheae are not included in this table.

Number of specimen	1		2		3		4		5		6		7		8		9		10		11	
	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L
Thorax	I	+ * -† +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +
	II	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +
	III	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +
Abdomen	I	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	II	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	III	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	IV	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	V	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	VI	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	VII	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	VIII	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
Total	8 + 6 = 14	10 + 3 = 13	5 + 9 = 14	8 + 5 = 13	8 + 5 = 13	8 + 5 = 13	8 + 5 = 13	8 + 5 = 13	8 + 5 = 13	6 + 5 = 11	6 + 7 = 13	5 + 8 = 13	7 + 5 = 12	8 + 5 = 13	5 + 5 = 10?	5 + 5 = 10?	5 + 5 = 10?	5 + 5 = 10?	5 + 5 = 10?	5 + 5 = 10?	5 + 5 = 10?	5 + 5 = 10?

* + = Trachea present. Perfect dissection in all but number 11

† - = Tube connecting left and right tracheae

TABLE III

TRACHEAE LEADING FROM THE LATERAL TRUNKS TO THE GANGLIA IN THE LARVAE OF *STRATIOMYIA LATIVENTRIS* LW.
Cephalo-prothoracic ganglionic tracheae are not included in this table.

Number of specimen	1		2		3		4		5		6		7		8		9	
	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L
Thorax	I	+ * - † +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +
	II	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +
	III	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +	+ - +
Abdomen	I	+ + ?	+ ? +	+ ? +	+ + +	+ + +	+ ? +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	II	+ +	+ +	+ +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	III	+ +	+ +	+ +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
	IV	+ +	? ?	? ?	+ +	+ +	+ ?	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
	V	+ +	? ?	? ?	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
	VI	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +
	VII																	
	VIII																	
Total	5 + 7 ? = 12?		Tissue injured in dissection		5 + 8 = 13		5 ? + 6 ? = 11 + 2 ?		7 + 5 = 12		7 + 5 = 12		5 + 5 = 10		5 + 7 = 12		7 + 5 = 12	
					Excellent specimen		Perfect example of no. VI on right		Perfect dissection		Perfect dissection		Good dissection		Poor specimen		Perfect specimen, perfect dissection	

* + = Trachea present † - = Tube connecting left and right tracheae

Anterior commissure and tubes to imaginal disks

Along the ventral side of the anterior end of the main trunks two or three pairs of short, branched tracheae (Fig. 6 d_1) lead to a pair of imaginal disks located caudad to the pharynx. From the median side of the main trunk, opposite the base of the first spiracular trunk, there is the second pair of tracheae (d_2) leading to imaginal disks. The anterior commissure (ac), a large, looplike tube connecting the main trunks, has its origin directly caudad from this pair of tracheae. Near the middle of the loop, but slightly separated, are two short tracheae (d_3), which lead to the third pair of imaginal disks craniad to the ganglia.

FUNCTION OF THE RESPIRATORY SYSTEM

RESPIRATORY CHAMBER

From the description and figures of the respiratory systems of certain *Odontomyia* and *Stratiomyia* larvae it is apparent that they breathe free air. The various parts of the respiratory system as well as the integument will be discussed in relation to respiration. Since the system has been described from the posterior end forward, the function of the parts of the system will be discussed in the same order.

Function of plumose hairs

The corona of plumose hairs surrounding the respiratory orifice is accessory to respiration rather than functioning, especially in the exchange of gases. It is possible that these hairs serve in a way similar to that of the integument, since they are hollow and are connected with the haemocoel. Mature larvae live and pupate, and adults emerge after the removal of plumose hairs, plainly showing that their primary function is not respiratory.

It has already been mentioned that these hairs are hydrofuge on the surface facing the orifice and that they spread funnel-like on the surface of the water while the rest of the larva is submerged. In this position the corona of hairs prevents water from entering the respiratory chamber and allows a free passage of gas to and from the respiratory system.

In addition to this important function in surface breathing these hairs are of great use to the larva when it is completely submerged. As a larva sinks the corona folds up, with the tips of the hairs coming

together to form a cone filled with atmospheric air. This store of air rests on the respiratory orifice and is available for respiration. As it is drawn into the respiratory chamber the cone collapses, and as gas is expelled from the tracheal trunks the cone forms again.

The mechanism for opening and closing the corona is not primarily muscular. As the orifice closes the hairs are brought nearer together at the apex of the funnel, but the corona will not fold up under this condition, when the larva is at the surface, nor will it open when the larva is submerged. The change in shape from a cone to a funnel is due to the flexibility of the shaft of each plumose hair. This was demonstrated in the following manner. The last somite, bearing a full complement of hairs, was clipped from an empty larval skin which had been dry for more than a year, and was thoroughly wet in distilled water. This structure floated on the surface even after it had been completely soaked. The dry hairs, which were matted together, gradually unfolded and formed a perfect corona. When this somite was forced below the surface, the corona folded up in normal fashion, taking a bubble of air down with it. When the somite was released, it came to the surface and the hairs spread out in the same manner as in living larvae. This was repeated a great number of times until the hydrofuge property of the hairs had been destroyed. With the loss of this property the hairs ceased to carry a bubble of air or to spread out on the water's surface.

The proportionate length of the hairs varies with the size and age of larvae of the same species. Mature larvae have shorter plumose hairs than do immature larvae. It seems that young larvae are much more dependent upon the hairs to maintain them at the surface of the water than are mature larvae. It is a well-known fact that these larvae drop to the bottom and rise again at will. In order that the larva may rise, gas is liberated from the respiratory system and held as a bubble by the plumose hairs. Eventually the bubble becomes large enough to float the larva to the surface. As soon as the tips of the hairs touch the surface they spread out. However, if the hairs are clipped off close to the body, especially in a young larva, the larva may hold sufficient gas in a bubble over the respiratory orifice to enable it to rise to the surface, but it drops back as soon as the bubble touches the surface.

Experiments were performed to test this phenomenon. In one the hairs were clipped from five larvae, and five similar larvae, un-

clipped, were used as controls. Test larvae and controls were placed in separate glass-stoppered bottles one third full of distilled water. To begin the experiment the bottles were shaken, and all larvae were submerged. In twenty minutes all the controls were either swimming on the surface of the water or crawling on the side of the bottle above the water line. The clipped larvae produced bubbles and one by one floated to the top only to have their bubbles explode, with the result that they dropped back to the bottom. It required twenty minutes for the slowest one to reach the top for the first time. Forty-five minutes after the experiment started one larva had repeated the trip to the surface. One hour after the beginning of the experiment a larva rose to the surface, lost its bubble of gas, and dropped back for the third time. It required twenty-five seconds for one of these larvae to float from the bottom of the container to the surface and three seconds to drop back the same distance. Test larvae repeated this phenomenon until they became exhausted, but the controls left the water and returned to it at will. This experiment was continued from April 11 to May 23 before all test larvae and controls were dead at the bottom of their bottles. They were without food through the experiment.

It is quite apparent that small larvae from which the plumose hairs have been removed cannot come to the surface and remain there. Mature larvae are not always affected in this way by the loss of the corona. The specific gravity of the larger ones seems to be just a little less, probably owing to the store of fat and oil or the large tracheal trunks filled with gas, or both. The plumose hairs of two large larvae of *O. cincta* were clipped July 28 and the larvae were still alive in the laboratory the following February 18.

Function of orifice

In the section on the morphology of the respiratory orifice it was made clear that the two chitinous blades forming the boundary of the orifice fit together perfectly when closed. When it is open, there is free passage of gas in and out of the respiratory system. In addition to closing the system to the passage of gas the orifice also prevents the entrance of water and other foreign matter. The surface of the blades is hydrofuge, as are the plumose hairs, and, in the absence of the hairs, does not allow water to creep over it into the respiratory chamber. This was demonstrated by clipping the hairs and submerg-

ing the larva by force. Very often a small bubble of air was held by the rim of the orifice.

The mechanism for opening and closing the orifice consists of a series of muscles inclosed in the last abdominal somite. These muscles work in conjunction with the respiratory chamber and will be discussed in detail along with the function of that organ.

Function of chamber

Gas passing through the orifice comes directly into the respiratory chamber, which serves as a passageway to the tracheal trunks and as a reservoir for respiratory gases. Since it is thin-walled and is supplied with muscles extending to various points on the inner surface of the integument, it is readily inflated and deflated. The semi-diagrammatic Figures 12-19 and 24 and the following description of the muscles will give some idea of how the respiratory chamber functions.

The first muscles, beginning with the posterior tip of the abdomen, arise from the ventral body wall, on either side of the anal groove, and extend obliquely toward the median line of the ventral wall of the respiratory chamber. These muscles (*v ms*) have a bifid insertion on the chamber and maintain this relative position caudad to the region of the posterior spiracles. In general, they are short, rather wide, and extremely thick. Their contraction tends to inflate the chamber.

A dorsal pair of oblique muscles (*d ms*) is similarly situated. The muscles are much shorter and narrower, and have the same action, probably contracting simultaneously to fill the chamber with gas.

Caudad to the spiracles a muscle extends across the haemocoel between the ventral body wall and the respiratory chamber. It is a straight muscle (*tr ms*) with a median attachment to the inner surface of the anal groove and lateral attachments on both the right and left ventro-lateral surfaces of the body wall. It intercepts the ventral oblique muscles and passes through them. Contraction of this muscle compresses the body ventro-laterally and partly opposes the muscles already mentioned.

A parallel pair of dorso-ventral muscles (*l ms*) extend across the lateral angles of the haemocoel. They are narrow but sturdy, and reach from the tip of the abdomen craniad to a point well in front of the spiracles. Their contraction compresses the body dorso-ventrally and closes the respiratory chamber.

There are at least three pairs of small muscles (*ob ms*), two of which extend from the dorso-lateral wall of the body to the dorso-lateral wall of the respiratory chamber. One pair in the region of the spiracles arise in the ventro-lateral wall of the body and are attached to the extreme lateral rim of the chamber. These muscles are very small. From their position it is apparent that they aid the dorsal and ventral muscles to inflate the respiratory chamber.

Another pair of important muscles (*bi ms*) arise at the tips of the small pouches in the cranial end of the chamber. Near their origin they bifurcate, with one half of each extending dorso-cranial to the roof of the haemocoel and the other half to the floor on either side of the anus. Contraction of these muscles compresses the chamber, pulls it forward, and aids in closing the orifice. The dorsal and ventral muscles work in opposition to these and pull the chamber back and allow the orifice to open.

Near the dorso-median rim of each spiracle there is a slender muscle (*lo ms*) which extends cranial to the dorsal body wall. In transverse section it appears like a longitudinal muscle, but it does not extend to the seventh somite for attachment. These muscles help to pull the chamber forward and act in conjunction with the muscles just mentioned.

Action of these muscles draws atmospheric air into the chamber and expels gas from it. Whatever part other muscles, not mentioned, may play in the inflation and deflation of the chamber, those described are the most important. It is believed that the process of external respiration is controlled in the region of the respiratory chamber. When the chamber is completely deflated, the inner dorsal and ventral surfaces rest against each other perfectly (Figs. 16-19), and the orifice is closed. The closing of the orifice, with the complete collapse of the chamber, forms a perfect seal for the gas inclosed in the tracheal system and prevents water from entering.

It is an easily demonstrated fact that larvae such as those used in this work liberate gas from the respiratory system while completely under water. This occurs when they are submerged by unnatural causes or by their own volition. A larva that is forcibly submerged carries a bubble of air with it. The bubble may be drawn completely into the tracheal system, or it may be enlarged by gas from the system and serve as a buoying agent to raise the insect to the surface again.

If a larva is robbed of its bubble of reserve air immediately after

it is forced down, it will eventually liberate gas to replace the air removed. Gas bubbles have been taken repeatedly from individuals without allowing them opportunity to replenish the supply from the atmosphere. There is, however, no regularity in the production of the bubbles. It varies under constant conditions, indicating that the larva controls this phenomenon.

Experiments were conducted with *O. cincta* and *O. virgo* to determine whether pressure applied to the body would force gas from the system. Pressure was exerted by placing the forefinger on the anterior end of a submerged larva and massaging toward the posterior end in an attempt to force gas from the tracheae through the spiracles and the respiratory chamber. Repetition of this act usually resulted in the yielding of gas, although the amount and regularity varied. Some larvae would not release any gas; some would release small quantities at irregular intervals; and others seemed to become fatigued under continued pressure and suddenly released large quantities of gas. Larvae that would not release gas when massaged were treated in the following manner. The tip of the abdomen was compressed laterally with forceps in order to cause the orifice to open. The point of a fine insect pin was inserted through the orifice and the compression relaxed. This pin in no way injured the larva, but kept the orifice slightly open while pressure was being applied. In some gas was forced out and in others it was not. This is proof that the orifice does not have complete control. An attempt to relax larvae by injecting chlorotone into the mesothorax had no effect on the controlling mechanism. That the writer might be certain that the specimens used in these tests did have gas in their tracheal systems each was bisected before it was allowed to come to the surface. In every one some gas still remained in the tracheae.

When the part of the somite containing the chamber and muscles had been snipped off, the gas was liberated freely, showing that control is due to muscular contraction in the region of the respiratory chamber. Prying the orifice open and compressing the chamber helped to counteract the action of the muscles to some extent. Under continued pressure and massaging these muscles relax, presumably to accommodate for the general discomfort of the larva.

The large pouches arising from the floor of the cranial end of the chamber are accessory gas sacs. They are continuous with the chamber and serve as extra storage space for respiratory gases during

extended periods of submergence. Experimentally they can be inflated and deflated along with the respiratory chamber. Since they are of softer membrane they collapse before the chamber does.

Function of the posterior spiracles

There can be no doubt that the spiracles in the cranial end of the respiratory chamber are the gates through which respiratory gases pass to and from the tracheal system. From their position it is obvious that gas must pass through them, but it was necessary to demonstrate how and through what part of the spiracle it goes. Since the spiracles are inclosed in the body and since the integument is opaque, it is impossible to observe the functioning of the spiracles in living larvae.

A technic was developed for the purpose of forcing air through the spiracles while they were submerged, and the results were ob-

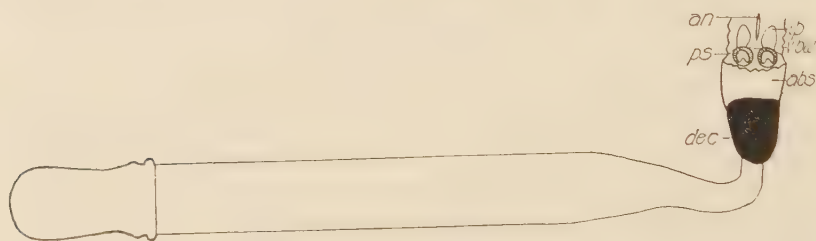


FIG. 21. Diagram of pipette, together with part of the last abdominal somite, showing the posterior spiracles exposed. Explanation of abbreviations: *abs*, last abdominal somite; *an*, anus; *dec*, De Khotinsky cement; *lp*, large air pouch; *ps*, posterior spiracle; *vbw*, ventral body wall

served with a binocular microscope. Other equipment, in addition to De Khotinsky cement and a dish of water, consisted of an ordinary pipette with the tip drawn into a capillary just small enough to be inserted through the orifice into the respiratory chamber.

The head and thorax of mature *O. cincta* larvae were suddenly dipped into boiling water for the purpose of killing them. Care was taken not to allow the last abdominal somite to touch the hot water. The capillary of the pipette was carefully prepared for insertion into the respiratory chamber. A thin coat of De Khotinsky cement was applied to the region of the capillary, which was to be sealed to the larva. In addition to the thin coat of cement a quantity sufficient to

complete the seal was added, so that it might be worked down the side of the capillary and completely cover the tip of the abdomen.

The last somite was compressed laterally, to force the orifice open, and the capillary was inserted. With hot probes and needles the cement was slowly warmed and worked over the tip until a perfect seal was effected. When there were no leaks the body of the larva was clipped off anterior to the anus. Pressure on the pipette bulb caused the air to pass through the spiracles and to bubble from the main trunks. (See Fig. 21.)

The next step consisted in exposing the anterior face of the spiracles without puncturing the respiratory chamber or the accessory gas pouches. A small lens knife was used under a binocular microscope. Desiccation was prevented by occasionally dipping the specimen in water.

Tests consisted of submerging the pipette and the attached dissection and observing with a microscope the effect of pressure on the bulb of the pipette. A steady gentle pressure caused the air to pass through the spiracles and form small bubbles. These bubbles emanated from the spiracles with a suddenness that made it difficult to detect the gas before the bubbles had formed or to determine the part of the spiracles through which it had passed. Pressure sufficient to force the gas through caused a spurt of bubbles which stopped as suddenly as it began when pressure was released. However, air could be seen along the ventral rim of the spiracle and between the chitinous rings and central membrane. Slight alternate pressure and relaxation on the bulb caused the air to pulsate between the short radiating tubes which form a grating from the ring to the membrane. The convincing evidence in support of the contention that this region of the spiracle functions in the passage of gas from the chamber to the trunks was apparent when a bubble was retracted through the spiracle into the chamber. Sometimes a bubble would remain in position as it formed and would be drawn back into the chamber as the pressure on the bulb was reduced. The bubble would diminish in size very gradually instead of suddenly, as it had formed originally. The last vestige of the bubble always disappeared through the grating of tubes between the ring and the membrane.

It has been demonstrated experimentally that gas passes through the ventral portion of the spiracles, and between the chitinous tubes rather than through them.

FUNCTION OF TRACHEAL SYSTEM

There is no question regarding the general function of the extensive system of tracheal trunks, branches, and fine ramifications. The controversial point involves that of carbon dioxide elimination in proportion to the oxygen received by the system. Regardless of the part played by the skin in elimination of carbon dioxide, the experiments which follow prove that a great deal of carbon dioxide finds its way into the tracheal system.

Respiratory movements

Since there is only one opening to the tracheal system through which gas can pass freely, there must be some mechanism for circulating the respiratory gases. Air enters and carbon dioxide passes out through the posterior spiracles. How the currents of gas move in the system is not known. Gas is kept in circulation, at least in part, by pulsations of the respiratory chamber. These pulsations are accompanied by the opening and closing of the respiratory aperture.

One larva, about ten millimeters in length, was observed under a binocular microscope. It rested with the corona spread on the surface of the water while the orifice opened and closed thirty-five or thirty-six times per minute. Opening and closing movements were correlated with a dorso-ventral compression and expansion observed only in the region of the respiratory chamber. The rest of the body may, and probably does, contract and expand, thus causing changes of pressure in the tracheae. The rate of pulsation was slightly accelerated by increasing the temperature of the water. This pulsating phenomenon is common when larvae rest at the surface, but is not a continuous process. It is believed that ventilation of the tracheal system is periodic rather than continuous.

Gas analysis

It has already been mentioned that *Odontomyia* and *Stratiomyia* larvae liberate from the respiratory system gas which it collects and holds as a bubble by the plumose hairs. If the hairs are removed the larvae liberate gas just the same, but are unable to hold it as well. The author took advantage of this habit of the larvae and collected samples of gas for microanalysis. It was not possible to obtain comparable results from the same larva or from a number of larvae

because there was no way to control the intake of air or the liberation of gas. Larvae may float at the surface of the water and not be breathing, or they may ventilate the system. Increased temperature stimulated their physical activity and also the respiratory exchange, as well as the liberation of gas. However, the same temperature did not produce the same rate of gas liberation from the same larva or from a series of larvae. The results, therefore, are of only relative significance.

All analyses were made with Krogh's apparatus (1908) for micro-analysis of gases, and according to his general directions. To facilitate reading the graduation marks the glass jacket around the graduated tube was filled with oil instead of water. When larvae were placed in the lower funnel of the instrument until bubbles of gas were liberated, the apparatus was sometimes charged with acidulated water to prevent absorption of carbon dioxide.

Table IV consists of selected examples of tests made under various conditions. With the exception of tests 8-13 the larvae were inserted into the lower funnel of the apparatus until gas was liberated. They were placed with the posterior end upward, so that the gas could be drawn into the capillary as fast as it was released. The results of tests 1 and 2 are not considered conclusive because of possible loss of carbon dioxide, which dissolved during the time required to collect a sufficient amount of gas for analysis. Charging the apparatus with 2.5 per cent and 5 per cent sulphuric acid was satisfactory, and the results were accurate. All the larvae used in this manner were treated first with acid to remove the calcium salts in the integument.

There seems to be no real correlation between the percentages of gases in the samples and the time required to obtain them; neither does there appear to be any marked ratio of carbon dioxide to oxygen in these samples. Test 6 yielded the highest percentage of carbon dioxide and a very low percentage of oxygen in one-half hour. There is no way of knowing how long the larva had retained the gas in its tracheal system without inhaling a fresh supply of air. The highest percentages were obtained from tests 6 and 7, collected over relatively shorter periods than tests 14-16. It is interesting to note that when the tracheal trunks of larvae that were free to breathe normally were snipped, gas was liberated which, when immediately analyzed, was composed of 1.8-7.4 per cent carbon dioxide and

TABLE IV
ANALYSIS OF GAS TAKEN FROM THE TRACHEAL SYSTEM OF *ODONTOMYIA CINCTA* LARVAE

Test no.	Medium for collecting gas	Temp. during test (Cent.)	Length of bubble (mm.)	Percentage		Remarks
				CO ₂	O ₂	
1	Distilled H ₂ O	21.6-21.9	11.2	5.48	1.78	Bubble robbed from larva and analyzed immediately
2	do.	21.9-21.9	12.8	6.27	4.67	Gas from two larvae
3	Pond water	21.6-21.9	82.5	7.63	1.09	Gas from one larva in 0.5 hour
4	do.	22.2-22.7	68.8	5.93	0.70	Gas from one larva in 1.5 hours
5	do.	21.1-21.5	35.8	2.08	0.90	Gas from one larva in 1.5 hours
6	do.	23.9-24.4	80.2	39.80	0.75	Gas from one larva in 0.5 hour
7	do.	24.7-24.4	88.4	38.91	0.83	Gas from one larva in 1.0 hour
8	2.5 per cent H ₂ SO ₄	21.9-22.2	70.4	5.24	2.97	Gas from one larva *
9	do.	22.7-23.3	72.8	4.93	3.68	Gas from one larva *
10	do.	24.4-24.1	63.8	3.13		Gas from one larva *
11	do.	21.1-21.4	86.5	4.07		Gas from one larva *
12	do.	22.2-22.2	69.7	7.46		Gas from one larva *
13	do.	21.1-21.1	54.7	1.83	0.55	Gas from one larva *
14	5.0 per cent H ₂ SO ₄	25.8-25.5	84.5	16.69	1.12	Gas from one larva in 4 hours
15	do.	27.7-26.9	75.8	23.75	4.29	Gas from one larva in 6.5 hours
16	do.	23.0-22.7	87.0	15.52	1.39	Gas from one larva in 21.5 hours

Determinations and calculations, including temperature corrections, were made according to Krogh's method (1908).

* Snipped with scissors and analyzed immediately.

3.6–0.5 per cent oxygen. The maximum oxygen content is lower for these tests than for test 15, in which the specimen was submerged for six and one-half hours.

These data show that larvae maintain in the tracheal trunks a relatively low percentage of oxygen and a high percentage of carbon dioxide. They also definitely establish the fact that a large amount of carbon dioxide eliminated by the tissues is collected by the tracheal system and expelled through the posterior spiracles.

FUNCTION OF THE ANTERIOR SPIRACLES

The anterior spiracles, situated dorso-laterally in the integument of the prothorax of the larva, have already been described morphologically. An effort has been made to determine the extent to which the spiracles are used in respiration. From structure alone it seems quite possible that air could be taken in very slowly through the stigma, pass through the "felted chamber," and then gain access to the main trunks. This would be a very slow process because the chambers are small and the mass of chitinous tubes in them forms a loose wad through which the air would necessarily have to pass.

Experimental results with mature larvae were not always comparable because it was not possible to know when all specimens used were in the same stage of development. Larvae often pupated during the course of the experiment, and the internal structures suddenly changed, while at the same time respiratory activities varied. Tests involving the closing of spiracles required relatively long periods of time because larvae with closed spiracles are able to survive a number of days on the gas held in the respiratory system.

The plan of testing involved closing the respiratory orifice with De Khotinsky cement and submerging the larvae either in kerosene or xylol containing Sudan III. It had previously been discovered that these oils stained with Sudan III entered the spiracles of young larvae and in a few hours displaced most of the gas in the tracheae. Since it passed through the spiracles of young larvae in a comparatively short time, it was supposed that it would enter the anterior spiracles of mature larvae if they were open and if sufficient time was allowed.

Larvae were thoroughly dried on blotting paper before the De Khotinsky cement was applied to the tip of the abdomen. The surface of the cement was softened little by little with the point of a

hot needle until the orifice was completely closed. This operation did not seem to harm the larvae and did produce a seal that was tight and not affected appreciably by kerosene or xylol.

The oils irritated the larvae and caused them to squirm and writhe vigorously. Their activity was watched with a binocular microscope to observe whether there was any release of gas from the anterior spiracles. Activity gradually ceased, and the larvae appeared dead. Those in kerosene were dissected and thoroughly examined 5.7 days after the test began. All the larvae were alive, but not a trace of Sudan III was found in the tracheal system. These larvae had been kept at ordinary room temperature throughout the experiment.

Larvae in xylol and Sudan III were slowly warmed to a temperature of 45-50 degrees centigrade. Their discomfort increased with the temperature. Again they were watched for the liberation of gas. It was supposed that if the respiratory orifice was sealed perfectly, and the anterior spiracles were open, the increase in temperature would cause the gas to expand and create sufficient pressure in the tracheal system to force the gas out through the anterior spiracles. At a temperature of approximately 50 degrees centigrade general activity ceased in 1.5 hours without liberation of gas from any part of the larvae.

The specimens were allowed to cool in the stained xylol for 50 minutes, during which time the temperature dropped to about 15 degrees and then gradually returned to room temperature, where it remained 4.5 hours. Two larvae were held in boiling water so that only the anterior half was submerged. The heat did not force gas out through the anterior spiracles of either, but when they were dropped on the boiling water gas was forced out of the posterior end. Bubbles were formed in the cement, which was softened by the hot water, indicating that the gas did not escape until the cement had melted. The melting took place so quickly that it was difficult to determine whether or not the cement had softened before any gas was liberated. In one case, at least, gas came through the soft cement and not between the cement and the integument.

Only one larva used in this severe test contained a trace of stained xylol. This slight amount of stain was found in the tracheae of the posterior end and most probably was due to a leak in the cement seal. The pressure tests seem to yield quite conclusive evidence that gas does not pass from the tracheal trunks out through the anterior spiracles of mature *O. cincta* larvae. Nor does kerosene pass in from

the outside. From the results of these experiments it seems fair to conclude that the anterior spiracles function very little, if at all, in the passage of respiratory gases.

RESPIRATION OF SUBMERGED LARVAE

Since these larvae are amphibious in their habits and spend considerable time beneath the surface of the water, they serve as excellent subjects for experiments in submergence. Preliminary tests consisted in placing single larvae in small stender dishes filled with water and submerging the dishes in a glass container. An equal number of controls were allowed to float on the surface of the water. After two days' submergence, half of the larvae were dead, and the other half were so affected that pupation and emergence of imagoes did not occur. Imagoes eventually emerged from two thirds of the controls.

Other tests indicated that larvae live longer when submerged in large quantities of water. This led the author to suspect either that they could use the dissolved oxygen in the water or that the water received something from them that made it toxic.

An attempt was made to determine experimentally whether larvae were able to use dissolved oxygen and give off carbon dioxide during forced submergence. A number were kept in bottles for varying periods, after which the water was tested for dissolved oxygen by the Rideal-Stewart modification of the Winkler method as given in *Standard Methods for the Examination of Water and Sewage*, by The American Public Health Association (1925). Carbon dioxide determinations consisted of titrations with N/44 potassium hydroxide in the presence of phenolphthalein as an indicator.

Boiled or distilled water that had been oxygenated was used in these tests. The supply of water was stored in a large carboy and siphoned into glass-stoppered bottles. A homogeneous mixture of the oxygen was obtained by thoroughly shaking the carboy before taking the samples. Test bottles were filled from the bottom and allowed to overflow two or three times the capacity of each bottle. Control bottles were filled in the same manner and at intervals during the filling of the test bottles. This precaution was taken for the purpose of detecting any change in dissolved oxygen content occurring in the water while the samples were being taken.

Larvae for these tests were generally brought in from the pools

the day before they were to be used. They were freed from organic matter, which usually clings to them, and dropped into 2.5 per cent hydrochloric acid to destroy small organisms and remove calcium salts in the integument. After the cleaning process they were left in a large volume of warm water for about 24 hours. The warmth made them physically active and stimulated evacuation of excreta.

As a test bottle was filled the larvae were suddenly introduced into the neck of the bottle with slender forceps, and the stopper was inserted. Sudden introduction of a larva prevented it from taking along a bubble of air. As soon as each bottle was filled with water and supplied with larvae, it was submerged in a large aquarium filled with water a few degrees cooler than the water in the bottles. This prevented leaking from expansion of the water in the bottles and likewise raised the solubility threshold so that the dissolved gases remained in solution. From time to time control and test bottles were removed from the aquarium and the contents tested for dissolved oxygen and carbon dioxide. In most instances bottles of 70 c.c. capacity were used for oxygen and those of 250 c.c. capacity for carbon dioxide determinations. It was generally quite simple to pick the larvae out of the bottles before adding the chemicals. Since the organic matter was considered negligible, the steps in the regular procedure previous to the introduction of MnSO_4 were omitted. The quantity of chemicals used was reduced in the proper proportion for 70 c.c. samples and the dissolved oxygen determinations were computed by titrating 50 c.c. of the sample.

The tests for dissolved free carbon dioxide were made by siphoning 100 c.c. of water into a Nessler tube from the test bottle. Phenolphthalein indicator was followed by titration with N/44 potassium hydroxide.

The data in Tables V and VI show that larvae are able to use dissolved oxygen from the water and give off carbon dioxide to it. In one instance twelve larvae removed all but a trace of dissolved oxygen from 70 c.c. of water in 62 hours and survived. This water had 3.7 c.c. of oxygen per liter when the larvae were put into the bottle. Most of the oxygen is removed in 18 hours. Records of tests in which there was any evidence of excreta were not included in the tables. It was originally supposed that the change in the amount of dissolved gas of the water was due to cutaneous respiration alone. Although larvae inclosed in the bottles did produce

TABLE V

DISSOLVED OXYGEN USED BY SUBMERGED LARVAE OF *ODONTOMYIA CINCTA*

The capacity of each bottle was 70 c. c.

Bottle no.	C. c. of dis. O ₂ per liter		Time in hours	No. of larvae	Temp. (Cent.)	Remarks
	Control	Test				
9	8.12		10	..	24.5-26.5	
4		7.67	10	5	do.	All alive
5		7.55	10	5	do.	do.
2	8.03		22	..	do.	
6		6.80	22	5	do.	do.
1	8.31		33.5	..	do.	
7		6.14	33.5	5	do.	do.
1	5.13		12	..	12.8	
2	5.13		12	..	do.	
4		4.90	12	5	do.	All alive
9		4.90	12	5	do.	do.
10		4.90	12	5	do.	do.
3	5.25		24	..	do.	
5		4.54	24	5	do.	do.
6		4.71	24	5	do.	do.
7		4.74	24	5	do.	do.
1	3.67		18	..	12.8	
2	3.64		18	..	do.	
3		3.49	18	5	do.	All alive
4		3.40	18	5	do.	do.
5		3.38	18	5	do.	do.
6		3.49	18	5	do.	do.
7		3.29	18	5	do.	do.
1	3.73		0	..	20-24	
2	3.73		15.5	..	do.	
6		2.13	15.5	10	do.	All alive
3	3.87		26	..	do.	do.
12		1.62	26	12	do.	do.
4	3.73		38	..	do.	
11		2.27	38	6	do.	do.
14		1.53	38	10	do.	do.
16		trace	62	12	do.	All recovered in 12 hours

TABLE VI

CARBON DIOXIDE ELIMINATION BY SUBMERGED LARVAE OF
ODONTOMYIA CINCTA

The capacity of each bottle was 250 c. c. The time of each experiment was 20 hours.

Bottle no.	CO ₂ in parts per million		No. of larvae	Temp. (Cent.)	Remarks
	Control	Test			
1A	4.0		..	22.2	
2A	3.5		..	do.	
3A	3.5		..	do.	
4A	...	17.5	7	do.	All alive
5A	...	11.0	7	do.	All very active
6A	...	9.0	7	do.	All very active
1A	7.0		..	24.6-26	
2A	5.7		..	do.	
3A	7.3		..	do.	
4A	...	11.0	7	do.	All alive
5A	...	13.7	7	do.	All alive. One pupa
6A	...	12.0	7	do.	All active

small bubbles occasionally, they did not do so as readily as under natural conditions. When bubbles were formed gas exchange took place at the surface of the bubbles. If cutaneous respiration occurred — and there is no doubt that it did — the results are included in these data.

Attempts to do qualitative analyses of respiratory gases were futile. During the summers of 1930 and 1931 larvae were obtainable in such small numbers that only a few series of tests could be made simultaneously. It was impossible to select larvae of exactly the same stage of development and with the same rate of gas exchange. It is necessary that they be of known genetic constitution before quantitative studies of metabolic processes can be attempted. This involves perfecting a technic for laboratory breeding and rearing under controlled conditions so that larvae from the same egg mass or a number of egg masses will develop uniformly. Until such technic is perfected the results of quantitative studies cannot be comparable and significant. This applies both to the respiration of submerged larvae and to the breathing of those in atmospheric air.

CUTANEOUS RESPIRATION

Cutaneous respiration has already been mentioned in connection with respiration of submerged larvae. The extent to which these larvae depend on the exchange of respiratory gases through the skin is not known. Acids, alkalies, alcohol, chloroform, and other chemicals apparently have little effect on these animals unless they enter the openings to the respiratory and digestive systems. The skin is quite impervious to these reagents and probably to oxygen and carbon dioxide.

Some preliminary experiments on gas diffusion through an empty larval skin were performed. The head and last abdominal somite were clipped from an active, mature larva, and the visceral organs and muscles removed as nearly completely as possible. The empty skin was allowed to dry. Small glass tubes were cemented to either end of the dry skin, and the tubes were passed through rubber stoppers. The stoppers served to support the skin and tubes in a glass jacket. The jacket was filled with either water or barium hydroxide, and the pressure regulated with a column of mercury. A three-way stopcock was connected to the free end of one of the glass tubes and a two-way stopcock to the end of the other one. Oxygen, or carbon dioxide, and mercury were admitted through the first stopcock, while the second allowed the excess gas to escape and mercury to be introduced.

After the jacket had been filled and the inner surface of the skin moistened with water the system was flushed with the desired gas and the pressure balanced inside and outside the skin. Mercury was introduced through the first cock and displaced the gas in the first tube almost to the skin. Mercury admitted through the second cock forced the gas in the second tube back through the skin into the first tube from which it displaced most of the mercury. The second cock was permanently closed and the first opened to the mercury reservoir. In this way oxygen or carbon dioxide was pocketed between two columns of mercury, with the larval skin as part of the system. One column of mercury was fixed in position; the other fluctuated as the volume of gas changed. There was no possible escape for the inclosed gas except through the skin. The position of the gas in the tube was marked, and the changes in the length of the column were recorded from time to time.

In every test of this kind the volume of inclosed gas decreased, an indication that both oxygen and carbon dioxide had passed, under slight pressure, through the larval skin from the inside out. The rate of diffusion was relatively constant throughout a test. The time for each test varied from 23 to 50 hours. In general, diffusion was more rapid at higher pressures. When barium hydroxide was used in the jacket the skin was examined for deposits of barium carbonate. If any was produced, it was not in sufficient quantity to be noticeable. Chemicals tests for it were not made. Neither was the water in the jacket tested for dissolved oxygen. Although gas diffused through the integument of dead larvae under experimental conditions, there is no positive assurance that it will pass through the skin of living larvae under normal conditions. The data from these experiments are inadequate to furnish conclusive evidence in favor of cutaneous respiration, but are merely suggestive.

SUMMARY

1. The data reported in this paper are the results of studies on the respiration and respiratory systems of the larvae of *Odontomyia cincta* Oliv., *O. virgo* Wied., *Stratiomyia discalis* Lw., *S. norma* Wied., and *S. lativentris* Lw.

2. Dissections were made in pure glycerine. Glycerine served as an excellent preserving fluid for showing the tracheal system inflated with gas.

3. Mature and young larvae of *O. cincta* and *O. virgo* overwinter in damp moss, decayed bark, and soft wood. *Stratiomyia* larvae were found in gravel above the water line of a river during freezing weather. Experimentally these larvae survived repeated freezing and thawing.

4. The skin is quite impervious to dilute acids, alkalies, alcohol, ether, chloroform, and oils such as kerosene and xylol.

5. Each posterior spiracle consists of a heavy chitinous ring within which a lighter ring rests. Both rings support chitinous tubes which radiate to a membrane in the central region. Gas passes between these tubes rather than through them.

6. The anterior spiracles possess "felted chambers," but apparently do not function as passageways for gas. Morphologically, it is possible for minute quantities of gas to pass through them, but this was not demonstrated experimentally.

7. The larvae used by the writer did not possess lateral spiracles on the metathorax and abdominal somites.

8. The respiratory chamber and accessory pouches serve as reservoirs for respiratory gases. Muscles extending from the body wall to the chamber open and close the orifice and depress and extend the chamber.

9. Young larvae depend on the plumose hairs to maintain themselves at the surface of the water. Mature larvae are less dependent upon the hairs for this purpose. The hairs are used to inclose bubbles of air either to float the larvae from the bottom to the surface of the water or to supplement the supply of oxygen in the tracheal trunks while the animal is submerged.

10. Larvae that are forcibly submerged are able to use dissolved oxygen from the water and to give carbon dioxide to it.

11. Analyses of gas liberated from the respiratory system as well as of that obtained by snipping the main tracheal trunks showed a great variation in the percentage of carbon dioxide and oxygen. The carbon dioxide content was always high and the oxygen content low as compared with that of air.

12. Cutaneous respiration in living larvae has not been proved although experimentally oxygen and carbon dioxide under slight pressure were noted to diffuse slowly through the body wall of dead larvae.

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KEY TO ABBREVIATIONS USED IN PLATES

- I-X* — attachment of spiracular trunks to main trunks
1-10 — dorsal connectives
a — spiracular trunk
abs — last abdominal somite
ac — anterior commissure
ag — accessory ganglionic trachea
agr — anal groove
an — anus
as — anterior spiracle
b — main visceral trachea
bi ms — bifurcated muscle
bl₁, bl₂ — ventral and dorsal blades of orifice
bw — body wall
c — minor visceral trachea
ca — canal in plumose hair
cav — cavity
cm — central membrane
cps — cephalo-prothoracic ganglionic trachea
d₁, d₂, d₃ — tracheae leading to imaginal disks
de — dermis
de c — De Khotinsky cement
d ms — dorsal muscle
e — "ear" of posterior spiracle
ep — epidermis
f — anterior branch of dorsal connective
g — ganglionic trachea
gt — minor connective
h — posterior branch of dorsal connective
hae — haemocoele
ht — main trachea to the head
hy — hypodermis
l — "lip" of posterior spiracle
l ms — lateral muscle
lo ms — longitudinal muscle
lp — large air pouch
lt — lateral trunk
ms — muscle from respiratory chamber to ventral body wall
mt — main trunk
ob ms — oblique muscle
p — pharynx
ph — plumose hair
pl — plumule
ps — posterior spiracle
r₁, r₂ — heavy and light chitinous rings
rc — respiratory chamber
ro — respiratory orifice
s — sternal trachea
sa — stigmatic aperture
sc — stigmatic or "felted" chamber
sh — shaft
sp — small air pouch
spi — spine
t — tergal trachea
tr — tricipora
tr ms — transverse muscle
tu — chitinous tubes in stigmatic chamber of anterior spiracle
tu₁, tu₂, tu₃ — chitinous tubes in the posterior spiracle
v bw — ventral body wall
v ms — ventral muscle

PLATE LXXX

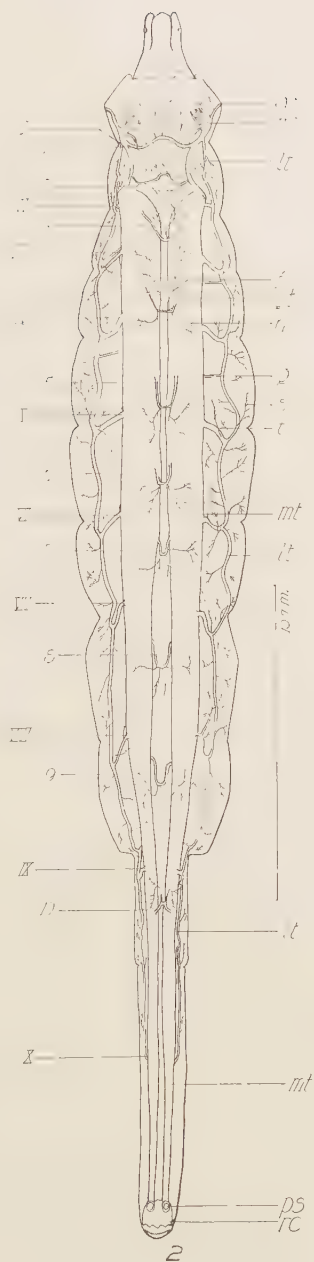
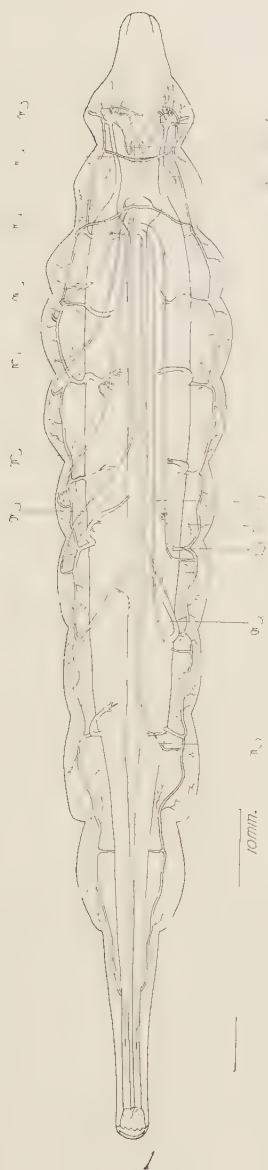


FIG. 1. Ventral aspect of the respiratory system of *Stratiomyia norma* Wied.
 FIG. 2. Dorsal aspect of the respiratory system of *Stratiomyia discalis* Lw.

PLATE LXXXI

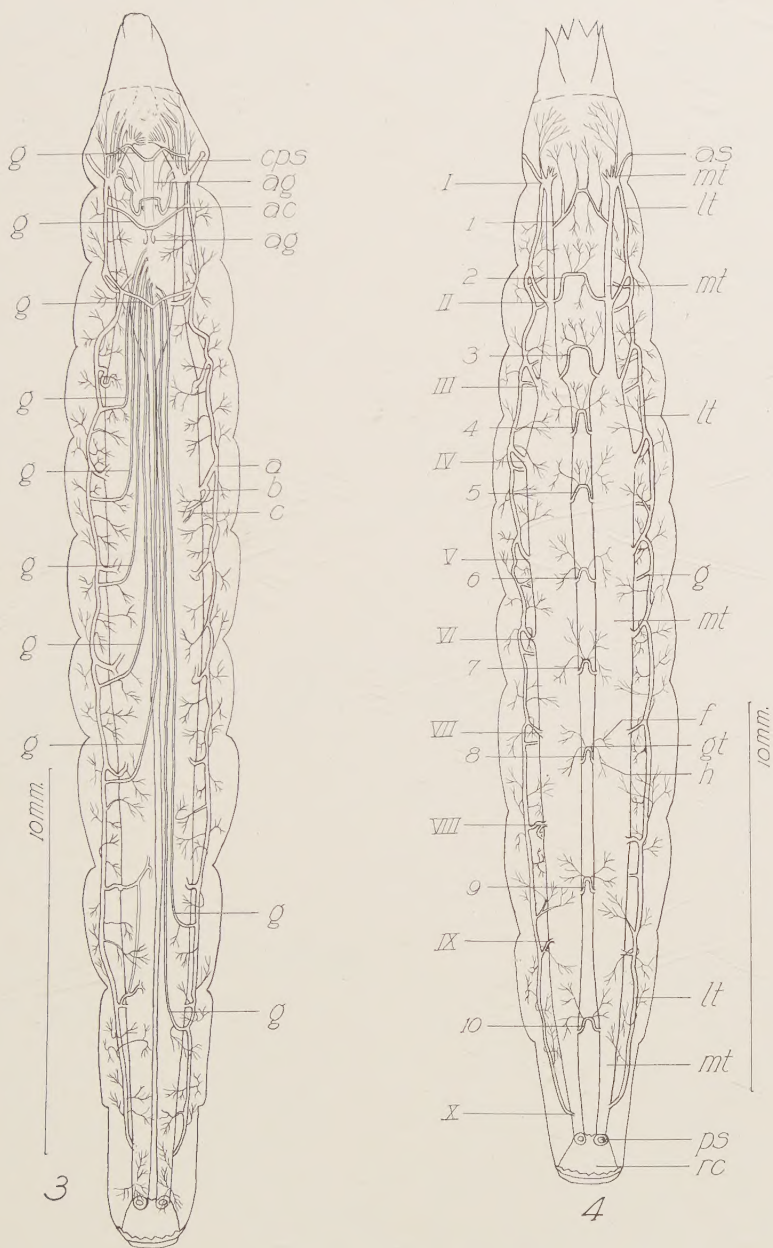


FIG. 1. Ventral aspect of the respiratory system of *Odontomyia cincta* Oliv.
 FIG. 2. Dorsal aspect of the respiratory system of *Odontomyia cincta* Oliv.

PLATE LXXXII

Odontomyia cincta Oliv.

FIG. 5. Diagram of the respiratory chamber, posterior spiracles, and main trunks

FIG. 6. Tracheae of the head and thorax

FIGS. 7-9. Sections through the anterior spiracle

FIG. 10. Cephalic portion of the posterior spiracle

FIG. 11. Caudal portion of the posterior spiracle

PLATE LXXXII

